








Antibacterial and antifouling potentials of ethanol-aqueous extracts of *Ericaria amentacea* collected from the northwestern coast of Algeria

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ABSTRACT

This work explored the antibacterial and antifouling properties of ethanol–water extracts from the brown macroalga *Ericaria amentacea*, collected along the northwestern Algerian coast. Extracts were prepared using maceration followed by ultrasonication. Antibacterial activity, assessed using the disk diffusion method, was most significant against *Escherichia coli* and *Listeria innocua*, with inhibition diameters measuring 20 ± 0.82 mm and 16.33 ± 0.46 mm, in that order, at a dose of 100 µg/mL. Moderate activity occurred against *Staphylococcus aureus*, *Listeria ivanovii*, and the yeast *Candida albicans*. In antifouling assays, the extracts significantly inhibited the growth of the diatom *Nitzschia* sp. at all tested concentrations, demonstrating a dose-dependent effect. The half-maximal effective concentration (EC₅₀) for the algal extract was 10.52 µg/mL, confirming potent bioactivity, although the synthetic biocide Diuron was more effective (EC₅₀ ≤ 5 µg/mL). These results position *E. amentacea* as a potential supplier of natural metabolites that can aid in producing eco-friendly antibacterial and antifouling agents.

Keywords: *Ericaria amentacea*, algal extracts, antibacterial activity, antifouling activity, northwestern Algerian coast.

INTRODUCTION

Brown macroalgae (Phaeophyceae) are vital components of coastal ecosystems and represent a promising resource for biotechnological applications. They form extensive underwater forests that serve as ecological hotspots, enhancing primary productivity, supporting biodiversity, and mitigating coastal erosion. Recent studies highlight their significant role in blue carbon processes, with kelp forests sequestering substantial amounts of CO₂ annually, often exceeding the capture rates of many terrestrial ecosystems (Eger et al., 2023; McHenry et al., 2025). This underscores their relevance in global carbon cycling, a point further emphasised by Qu et al. (2023).

Marine macroalgae are valuable reservoirs of biologically active substances that can be

exploited in fields such as pharmaceuticals, nutraceuticals, cosmetics, and environmental biotechnology (Pérez et al., 2016; El Hattab, 2020). Brown algae, in particular, have attracted growing significant scientific interest because of their distinctive chemical diversity and broad spectrum of biological activities. They synthesize numerous secondary metabolites, including phenolic derivatives (e.g., phlorotannins), terpenoids, polysaccharides, and fatty acids. These compounds play crucial ecological roles in defense against pathogens, herbivores, and biofouling organisms, while also holding considerable promise for biotechnological exploitation (Negara et al., 2021; Khan et al., 2022).

Within this group, species of the genus *Ericaria* are recognized to be abundant producers of diverse secondary metabolites exhibiting antimicrobial, antioxidant, and antifouling properties

(Oddo, 2020; De la Fuente et al., 2022). Their ecological and bioactive potential has been corroborated by recent investigations (Radman et al., 2022; Bouafir et al., 2025). *Ericaria amentacea* (C. Agardh) Molinari and Guiry, a dominant macroalgal taxa distributed along Mediterranean coasts (Thibaut et al., 2014; Bordoni et al., 2025), is increasingly studied for its ecological role and chemical composition. However, investigations into its bioactive potential, particularly concerning antibacterial and antifouling effects, remain limited, especially for populations inhabiting the western coast of Algeria.

The present work focuses on characterizing the antibacterial and antifouling potential of ethanol–water extracts obtained from the brown macroalga *Ericaria amentacea* harvested from the Algerian western coast. Specifically, it investigates the inhibitory effects of these extracts against selected microbial strains and assesses their impact on the growth of the marine diatom *Nitzschia* sp. The research also quantifies extraction yields and determines the half-maximal effective concentration (EC_{50}) to benchmark the bioefficacy of the algal extracts against that of a commercial biocide, Diuron. The findings aim to substantiate the potential of *E. amentacea* as a source of natural, eco-friendly compounds for antimicrobial and antifouling applications. The sustainable cultivation of this alga is proposed as an essential strategy to prevent the over-exploitation of this vulnerable species.

MATERIALS AND METHODS

Algal material and preparation

In March 2021, thalli of *Ericaria amentacea* were sampled from the western coastal zone of Algeria at a depth of one meter. The holdfast was left in place to encourage regrowth and minimize the impact of collection; only the apical and middle portions of the thalli were sampled. Plastic bags containing marine algae were stored on ice. Species identification was conducted using standard Taxonomic keys, with subsequent verification against the Algaebase database (www.algaebase.com), within the Laboratory of Aquaculture and Bioremediation. The samples underwent repeated rinsing in the lab with sterile seawater to eliminate sand particles and epiphytes. After two days of shade drying at room temperature (25 °C),

they were further dried for 24 hours at a low temperature (30 °C) in an air circulating oven. Before further analysis, the samples were milled into a fine particulate and preserved at +4 °C.

Protocol for algal extract production

Extracts were prepared at serial concentrations (5 µg/mL, 10 µg/mL, 20 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL). 50 g of dried matter brown algae was added and soaked in 100 mL of an ethanol-distilled water mixture (75:25) at 200 rpm for 3 hours protected from light at ambient temperature. Ultrasonication was performed with a Sonopuls HD 2070 homogenizer equipped with a UW 2070 converter (Bandelin Electronic GmbH & Co. KG, Berlin, Germany) at a fixed frequency of 20 kHz. Following ultrasonication (35% amplitude, 30 min), the mixture was spun at $5000 \times g$ for 30 min at 4 °C (Sigma 3-18K). The clear phase was separated via filtration (Whatman No. 1), after which the liquid fraction was concentrated under reduced pressure at 40 °C (Hahn timer HS-2005-N). The dried residues were dissolved in 2% DMSO, sterile-filtered (0.22 µm), and held at -20 °C.

Yield

The yield of the dry extracts is calculated using the following formula:

$$R (\%) = (P1 - P2) / P3 \times 100 \quad (1)$$

where: $P1$ – the balloon's weight following evaporation; $P2$ – the flask's empty weight prior to evaporation; $P3$ – dry algal material weight.

Test microorganisms

Extracts of *E. amentacea* were tested at various concentrations against microorganisms: *Escherichia coli* (ATCC 22000), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Listeria innocua* (ATCC 33090), *Listeria ivanovii* (ATCC 19119), *Candida albicans* (ATCC 26790) (provenance Algerian Pasteur Institut collection of AQUABIOR), and the diatom *Nitzschia* sp. *B10f-3* (Nantes Culture Collection, France).

Diffusion agar test

The antimicrobial activity of the derived solutions was assessed using the standard disc

diffusion assay, following established protocols (Balouiri et al., 2016). The test bacteria were standardised to a concentration of 10^8 CFU/mL (0.5 McFarland). Sterile 6 mm discs (Whatman No. 1) were loaded with 30 μ L of each extract, with 2% DMSO serving as a negative control. The treated discs were then placed on Mueller-Hinton agar plates that had been swabbed with the bacterial suspensions. Post-incubation at 35 °C for 24 hours, the resulting halo sizes were measured in millimetres.

Culture of microalgae

Diatoms were aseptically cultured in a simplified seawater-based F/2 medium at 22 °C, with the media replaced every 15 days (Asfour et al., 2017). Artificial seawater was synthesized from NaCl (30 g L⁻¹), MgCl₂ (10.2 g L⁻¹), and KCl (0.74 g L⁻¹), sterilized, and then enriched with Guillard's F/2 medium. The final culture medium was stored at 4 °C until required.

Evaluation of the activity of *Ericaria amentacea* extracts on the growth of microalgae

A volume of algae inoculum at a concentration of 10^5 cells per mL is spread on petri dishes containing a nutrient medium with varying concentrations of extracts (only concentrations showing effects were tested) or biocides. It is incubated at 22 °C under a natural light cycle. The effect is assessed using the method defined by Silkina et al. (2012), modified.

Seventy-two hours later, the effect of the extracts is evaluated by measuring chlorophyll a concentration through spectrophotometry. Post-extraction in methanol (99.8%, Sigma-Aldrich), supernatant absorbance was read at 652 and 665 nm to assess pigment concentration. These optical readings were then used to quantify chlorophyll a according to equation (2) (Qin et al., 2021). Cell counts are performed daily for three days using a Malassez-type hemacytometer under a microscope. On the fourth day, the cells are centrifuged at 2000 rpm for 10 minutes at 20 °C (sigma 3-18k). The culture medium is removed, and the cells are transferred to F/2 medium. Cell growth is then monitored daily for 8 days. The EC₅₀ (concentration inhibiting 50% of algal growth) is calculated and expressed as a percentage of inhibition.

$$\% \text{ of inhibition} = \frac{(Chl-a_c - Chl-a_t)}{Chl-a_c} \times 100 \quad (2)$$

where: $Chl-a_c$ corresponds to the chlorophyll a concentration of the control; $Chl-a_t$ corresponds to the chlorophyll a concentration of the strain tested in the presence of a given concentration of extract.

Synthetic biocide

A stock solution of the poorly water-soluble biocide diuron (N-[3,4-dichlorophenyl]-N, N-dimethylurea) was prepared using an organic solvent. Dimethyl sulfoxide (DMSO; 99%, Sigma-Aldrich) was selected as the carrier solvent based on established protocols, as it is miscible with water and has been reported to exhibit low toxicity towards diatoms (Bazes et al., 2009). A master stock solution of diuron was first prepared at 10 mg/ml in DMSO. Serial dilutions in sterile, double-distilled water were used to bring this stock into the culture medium, ensuring a final DMSO concentration of 0.2% (v/v) in all test solutions, as per a protocol adapted from Silkina et al. (2012).

Statistical analysis

Data are presented as mean \pm SD (n = 3) for antibacterial tests and as duplicate measurements for inhibition of diatom tests. The statistical analyses were performed using Microsoft Office Excel 2016.

The concentration resulting in 50% growth inhibition (EC₅₀) was determined by linear regression in GraphPad Prism (v.10). Reported EC₅₀ values are constrained to the experimental concentration range.

RESULTS

Yield

Ethanol aqueous *E. amentacea* extracts yield was $2.14 \pm 0.42\%$.

Antibacterial and antifungal assay

Table 1 shows that extract activity against bacteria and fungi depends strongly on the micro-organism and the concentration applied.

Effect on Gram-negative bacteria

The inhibitory zone diameter of *E. coli* grew steadily with increasing extract concentrations, from 8 ± 0.82 mm at 5 $\mu\text{g/mL}$ to 20 ± 0.82 mm at 100 $\mu\text{g/mL}$. However, *P. aeruginosa* exhibited complete resistance at all tested concentrations.

Effect on Gram-positive bacteria

L. ivanovii and *L. innocua* showed sensitivity to the extracts, with inhibitory zones increasing as concentration increased. While *L. ivanovii* exhibited reduced sensitivity, starting at 6 ± 0.82 mm at 20 $\mu\text{g/mL}$ and reaching only 9.33 ± 0.94 mm at 100 $\mu\text{g/mL}$, the results for *L. innocua* ranged from 9.33 ± 0.94 mm at 5 $\mu\text{g/mL}$ to 16.33 ± 0.46 mm at 100 $\mu\text{g/mL}$.

Staphylococcus aureus showed resistance at low to moderate concentrations, with inhibition observed only at the highest concentrations (9.33 ± 0.47 mm at 50 $\mu\text{g/mL}$ and 11 ± 0.1 mm at 100 $\mu\text{g/mL}$).

Antifungal assay

At doses of up to 25 $\mu\text{g/mL}$, *Candida albicans* remained resistant; however, at doses of 50–100 $\mu\text{g/mL}$, inhibitory zones measuring 7 ± 0.1 mm and 8.67 ± 1.24 mm appeared.

Effect of *E. amentacea* extracts and diuron on the growth of *Nitzschia* sp.

The growth curves of *Nitzschia* sp. in the presence of *E. amentacea* extracts at various and efficacious doses (10, 20, 25, 50, and 100 $\mu\text{g/mL}$) and the reference biocide diuron are shown in Figure 1. In the control treatment, algal cell density steadily increased, reaching 270×10^5 cells/mL by day 8. In contrast, all concentrations of *E. amentacea* extracts significantly inhibited algal growth relative to the control. The level of

inhibition was concentration-dependent: at the minimum tested dose (10 $\mu\text{g/mL}$), the inhibitory effect was minimal, with algal density reaching 250×10^5 cells/mL. At 20 $\mu\text{g/mL}$, growth was moderately reduced (238×10^5 cells/mL on day 8), whereas stronger inhibition occurred at 50 and 100 $\mu\text{g/mL}$, with final cell densities of 180×10^5 and 175×10^5 cells/mL, respectively. However, this inhibitory effect was lower than that of the reference biocide, which almost completely suppressed algal growth throughout the experiment. This was confirmed by the calculated EC_{50} value of 10.52 $\mu\text{g/mL}$ for *E. amentacea* extracts. In contrast, diuron exhibited a much lower EC_{50} value (≤ 5 $\mu\text{g/mL}$).

On the other hand, we notice that once transferred to a new medium, the microalgae cultures exposed to brown algae extracts recover more slowly than the control culture, unlike those exposed to the commercial biocide, which do not return to a normal growth curve, demonstrating the toxic strength of the latter.

DISCUSSION

In this study, maceration, ultrasound, and centrifugation procedures using a green solvent mixture (ethanol-water) were employed to enhance overall yield and bioactive compound levels from *E. amentacea* (Azmir et al., 2013; Castillo et al., 2023). Recent works have reinforced the efficiency of ultrasound- and microwave-assisted techniques as advanced approaches for optimizing the isolation of bioactive compounds (Taherkhani et al., 2024).

The antibacterial test showed that the plant extracts exhibited good activity against *E. coli* (20 ± 0.82 mm) and *L. innocua* at 100 $\mu\text{g/mL}$ and moderate inhibition on *S. aureus* (11 ± 0.1 mm) and *L. ivanovii* (9.33 ± 0.94 mm) at the same concentration.

Table 1. Antibacterial and antifungal activity of ethanol–aqueous extract of *Ericaria amentacea*

Concentration ($\mu\text{g/mL}$)	<i>E. coli</i>	<i>L. innocua</i>	<i>L. ivanovii</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>C. albicans</i>
5	8 ± 0.82	9.33 ± 0.94	R	R	R	R
10	8.33 ± 0.94	11 ± 0.82	R	R	R	R
20	9.67 ± 0.47	11.67 ± 1.25	6 ± 0.82	R	R	R
25	10 ± 0.10	12.67 ± 0.94	6.33 ± 0.46	R	R	R
50	14.33 ± 0.47	13.67 ± 0.47	9 ± 0	R	9.33 ± 0.47	7 ± 0.10
100	20 ± 0.82	16.33 ± 0.46	9.33 ± 0.94	R	11 ± 0.10	8.67 ± 1.24

Note: Zones of inhibition are expressed in mm \pm SD. R = resistant (no inhibition).

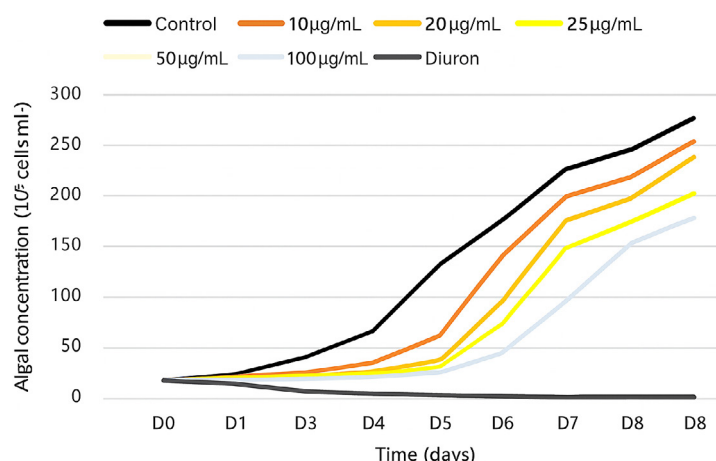


Figure 1. Growth response of *Nitzschia* sp. under exposure to increasing concentrations of *Ericaria amentacea* extracts and diuron (mean \pm SD, $n = 2$)

However, no inhibitory effect was observed against *P. aeruginosa*. *C. albicans* yeast displayed the greatest inhibition at 100 $\mu\text{g/mL}$, with only 8.67 ± 1.24 mm. De la Fuente et al. (2022) reported results with the methanol/dichloromethane extract of *E. amentacea*, showing 87% inhibition against *S. aureus* and 27% against *C. albicans*.

The resistance exhibited by *P. aeruginosa* likely results from its lipopolysaccharide-rich outer membrane, which hinders the penetration of molecules, including antibiotics (Piacenza et al., 2017). Maggio et al. (2020) specified that the methanol-water extract of *E. amentacea* inhibited the Gram-positive strain *S. aureus* but had no effect on the Gram-negative strain *E. coli*. The same author mentioned that this species contained the highest amount of phenol, particularly fukiic acid and bromophloroglucinol, which may be responsible for this activity. Yong-Xing Li et al. (2011) noted that the antibacterial efficacy of phlorotannins is influenced by factors such as molecular weight, and Wang et al. (2009) revealed that their antibiotic effect may be both bactericidal and bacteriostatic. Mirata et al. (2023) and Radman et al. (2022) also documented that Mediterranean populations of *E. amentacea* are rich in phenolic compounds, which confer antioxidant and photoprotective properties. The levels and bioactivity of these metabolites are strongly shaped by environmental parameters, such as light, temperature, and nutrient supply (Lopes et al., 2024). Mannino et al. (2016) observed a seasonal trend in total phenolic compounds, with peak levels occurring during winter and spring in *E. amentacea*.

Oucif et al. (2017) observed a phenolic content of 8.24 ± 0.89 and 4.36 ± 0.21 mg GAE g^{-1} DW in the methanol and ethanol extracts of *E. amentacea* collected on the west coast of Algeria. This alga is considerably richer in antioxidant compounds compared with TPC content (De la Fuente, 2020). In contrast, the ethanol extract of the brown algae *Cystoseira compressa* inhibited *S. aureus* (29 ± 0.00) but did not affect *P. aeruginosa* (Leibirsh et al., 2020). Tajbakhsh et al. (2011) mentioned that a combination of diethyl ether, ethanol, and hexane extracted from *C. trinodis* stopped the expansion of *E. coli*, *S. aureus*, and *P. aeruginosa*.

Sayin et al. (2021) recorded that the methanol extract of *E. amentacea* exerts both inhibitory and bactericidal effects toward *E. coli*, *S. aureus*, and *P. aeruginosa*. Our results align with those revealed by Erturk et al. (2011) and El Wahidi et al. (2015), who used ethanolic extracts of *Padina pavonica*, *C. barbata*, and *C. compressa* to test against *S. aureus*, *E. coli*, *L. monocytogenes*, and *C. albicans*.

Lopes et al. (2013) extracted phlorotannins from *C. nodicaulis* using hexane, followed by an acetone-water mixture, and observed an inhibitory effect against *C. albicans* (MIC = 15.6). Conversely, Vimala and Poonghuzhali (2017) noted that the ethyl acetate extract of the brown algae *Hydroclathrus clathratus* demonstrates the maximum activity against *C. albicans* (18.14 ± 1.50 mm). Cox et al. (2010) found that methanol extracts from various brown seaweeds inhibit *L. monocytogenes* and *P. aeruginosa*.

Extraction solvents play a vital role in isolating compounds from algae. Ethanol and its aqueous mixtures are the most suitable solvents for

extracts targeted for use in pharmaceutical, food, or cosmetic industries (Generalic-Mekinic, 2019).

The yields of obtainable and antibacterial compounds from several seaweed species vary depending on the solvent used (Pérez et al., 2015). Some studies suggest that polar extracts generate extracts with enhanced antibacterial effects. Yoon et al. (2017) found that edible brown seaweed *E. cava* produced the highest amount of phlorotannins when extracted with 95% ethanol. Other research confirms that combinations of hydrophilic solvents and/or alcohol-based solutions offer improved efficacy (Krish et al., 2014; De Jesus Raposo et al., 2015). Honey et al. (2024) reported that using multiple solvents of varying polarities for the same extraction would likely increase the antimicrobial inhibitory effect.

The antimicrobial activity of algae can be shaped by a range of factors, notably habitat, developmental stage, algal conditioning, extraction method, season of collection, activity of biofoulers and grazers, and pollution (Silva et al., 2020; Emeline et al., 2021), among others, which may explain the differences in findings reported by different researchers. Brown algae were the most extensively studied macroalgae. Their effects were primarily antibacterial, including anti-quorum-sensing, with additional activities such as anti-algal, anti-diatom, and anti-larval actions (Dahms et al., 2017; Lemesheva et al., 2023).

In our study, *E. amentacea* significantly reduced *Nitzschia* growth at all tested concentrations without causing any harmful effects, with an EC_{50} of 10.52 $\mu\text{g/mL}$. In contrast, diuron shows irreversible inhibition of diatom growth at the minimum tested dose (5 $\mu\text{g/mL}$), preventing the determination of its EC_{50} and confirming its extremely high toxicity. Moraes et al. (2019) indicated that a widespread inability to pick new drug candidates should have greater than 50% inhibitory activity at a concentration below 30 μM .

The majority of the activity-bearing active components were uncharacterised; however, they were mainly isolated with polar alcohol-based extracts (Negara, 2021). The methanol extract of the brown algae *C. mediterranea* showed significant antifouling activity. The main compounds in this extract were fatty acids and steroids (Ibrahim et al., 2019). Therefore, Bazes et al. (2009) indicated that palmitic acid, which is commonly found in algae, exhibits notable inhibitory activity against the diatom *Closterium* ($CE_{50} = 45.5 \mu\text{g/mL}$). Oucif et al. (2019) reported a palmitic acid content of $28.70 \pm$

0.74 g/100 g in *E. amentacea* from Algeria's west coast. Silkina et al. (2012) confirm the sensitivity of diatoms (*F. pinnata*, *C. closterium*, and *T. pseudonana*) exposed to ethanol extracts of a pheophyceae (*Sargassum muticum*) and a rhodophyceae (*Ceramium botryocarpum*), with growth EC_{50} values of 4.74 and 5.3 $\mu\text{g/mL}$, in that order, and a temporary effect on diatom cell division. The authors indicate that biocides were more effective ($EC_{50} = 0.52 \mu\text{g/mL}$) but potentially toxic.

CONCLUSIONS

Marine seaweeds are increasingly recognized as sources of biologically active compounds. Extracts from *E. amentacea* demonstrate antibacterial, therapeutic, and antimicrobial properties. They effectively inhibit *E. coli* and *L. innocua*, while showing moderate activity against *S. aureus*, *L. ivanovii*, and *C. albicans*. Conversely, *P. aeruginosa* was resistant.

All tested concentrations of *E. amentacea* significantly inhibited algal growth compared to the control. However, this inhibitory effect was reversible and less effective than the reference biocide, which nearly eliminated algal growth throughout the experiment. Although less potent than diuron, *E. amentacea* extracts provide a promising eco-friendly alternative because of their biodegradability and likely reduced harm to non-target organisms.

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